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The Pigeon as a Subject in Studies of Laser-Induced Retinal Damage: Histological Studies

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The natural history of laser-induced retinal lesions in the pigeon eye is reported, and the pigeon is proposed as a reasonable animal model for studies of laser-induced damage in primate eyes. Energy levels of approximately seven times the ED_{50} for an ophthalmoscopically observable retinal lesion were placed with a multiwavelength argon laser. Each lesion was induced by a one-second exposure at a power of 48 mW incident on the cornea. The bulk of the damage occurred in the photoreceptor outer segment and retinal pigment epithelium layers. Over the course of one week, edema and mild disorganization spread into the inner parts of the retina; at 1 day the inner nerve fiber layer was edematous; at 8 days it was homogeneous in the region immediately above the area of maximum damage. The presence of oil droplets in the pigeon retina did not appear to alter the response of the eye to the damaging effects of the laser. The present study was performed to provide baseline data so that pigeons may be more readily used in the future. The results obtained here imply that the pigeon can be a useful experimental subject and that, within the limits of our experimental design, the differences in structure of the pigeon retina do not prevent data obtained in that species from contributing to the understanding of basic mechanisms of laser-induced retinal damage in humans.

KEYWORDS: *laser, retina, pigeon, histopathology, ocular hazards*

Lasers, by virtue of their unique characteristic of coherency, produce minimal spot irradiation of the retina. These minimal spot exposures are able to selectively destroy retinal tissue or temporarily inhibit portions of the retina from processing and coding visual stimuli. The most important portion of the retina, the fovea, can be irradiated through an on-axis exposure in an anesthetized subject or when an awake and behavior-conditioned animal directly views the laser source.

The use of primates has historically precluded any immediate or systematic morphological and histological assessment of the laser-induced anatomic changes. An ideal research subject would be a diurnal animal that is highly dependent on vision, is inexpensive, easy to maintain, and which has a well-developed database for visual work, such as the pigeon.

The present studies were undertaken to establish a baseline set of anatomic data in an organism (the pigeon) which will be easier to maintain and handle than are primates and in which both behavioral and histopathologic studies can be undertaken using a single animal. These studies were concerned only with histopathologic changes in the lesion and the natural history; psychological and visual function studies will be reported at a later time.

MATERIALS AND METHODS

Six male white carneaux pigeons were used in this study. In preparation for exposure, each pigeon was sedated via sodium pentobarbital (Nembutal) (25 mg/kg) IM, and anesthesia was induced via ketamine (50 mg/kg) IM. Pupils were dilated with d-tubocurarine solution applied topically and sutures were placed in the lower eyelid so that the eye could be held open during exposure. The surface of the eye was irrigated with normal saline at approximately 15 second intervals throughout the course of the experiment. Twelve or sixteen nominally identical lesions were placed in an array in the retina of the left eye of each pigeon using a multiwavelength argon laser. Each lesion was induced by a one second exposure at a power of 48 mW incident on the cornea. This dose was seven times the ED50 for an ophthalmoscopically observable lesion (as determined in our laboratory). The incident laser beam was collimated so that diameter of the laser beam on the retina was governed by the optical quality of the pigeon eye. The retinal beam diameter for these exposures is estimated to be about 30 micrometers. Eighty percent of the power of the argon laser was emitted in approximately equal parts at wavelengths of 488 nm and 514.5 nm. The remainder was distributed amongst the wavelengths of 502 nm, 477 nm, 465 nm and 457 nm.

At 5 minutes, 1 hour, 1, 2, or 8 days after exposure, the animal was sacrificed by injection of T-61 euthanasia solution. The eyes were immediately removed, opened immediately posterior to the ora serrata, and placed in Karnovsky's fixative [1] which had been diluted with one volume of distilled water and 3.5 volumes of 0.2M Na cacodylate (final pH 7.4, 550 mOsm). Specimens were allowed to fix at least 24 hours. For each eye, the area with the lesions was located and removed with the aid of a dissecting microscope. Tissues were processed as previously described [2] and embedded in Spurr's plastic [3]. Each specimen was sectioned with a glass knife at 2 micrometer thickness. The sections were stained with methylene blue-azure II and basic fuchsin [4].

RESULTS

The specimen taken 5 minutes after exposure to the laser (Figure 1) was characterized by superficial vacuolation and homogenization of the nerve fiber layer between ganglion cell nuclear layer and innermost retinal boundary with edema and rarefaction in the layer between the outer nuclear layer and the ganglion cell bodies. There was pyknosis of the inner and outer nuclear layers with disruption of the outer limiting membrane. Photoreceptor cells were absent in some areas, and the outer nuclear layer was occasionally displaced to a position adjacent to Bruch's membrane. Peripheral to the area of maximum damage, the melanin of the retinal pigment epithelium was denser, disorganized and displaced inward. In some sections of these lesions, the photoreceptor outer segments appeared to have undergone coagulation. In this specimen is shown damage due to the incident laser beam per se; subsequent sections show secondary damage due to edema, cell death, and the beginnings of attempts at repair.

For example, specimens taken 1 hour after exposure to the laser (Figure 2) were characterized by edema and rarefaction of the nerve fiber layers extending to the innermost boundary of the retina. This disruption was seen to varying degrees through different sections through the eye. In some regions, vacuolation and pyknosis of the inner nuclear layer is seen. In occasional sections, a single, discrete, well-circumscribed vacuole at about the position of the outer limiting membrane was seen. In other sections, the vacuolation was

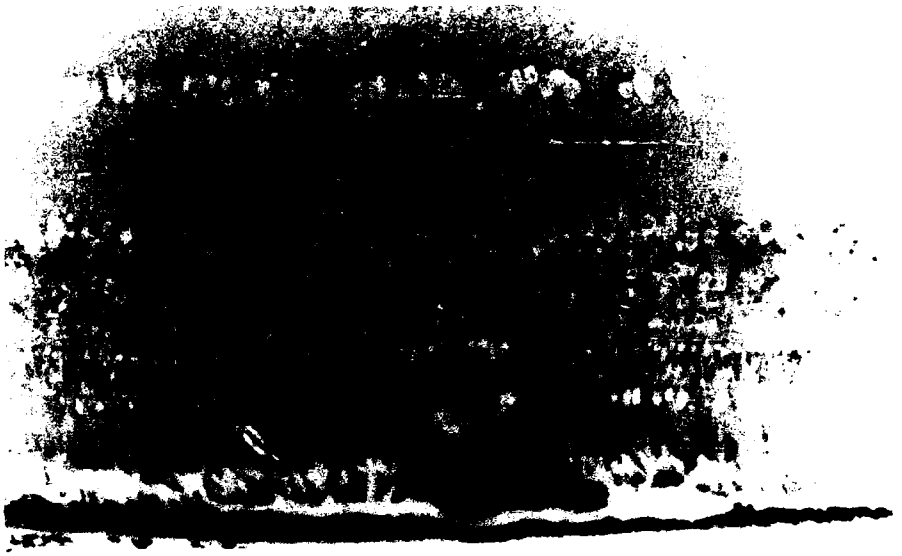

FIGURE 1 Section through pigeon retina harvested 5 minutes after exposure to an argon laser beam at 7 times the ED_{50} for an ophthalmoscopically visible lesion. Note pyknosis of outer nuclear layer and severe damage to retinal melanin layer and photoreceptor layer. Two micron section, original magnification 125×.

marked, about 15 cells wide; the cavity of the vacuole was sometimes occupied in part by cell debris and pyknotic nuclei; in many of the sections, the cavities are empty. There was marked vacuolation on both sides of the outer limiting membrane, involving both the inner and outer nuclear layers. In the areas of maximum damage, the retinal pigment epithelium was disrupted. In more peripheral areas of the lesion, the melanin layers of the retinal pigment epithelium were disorganized without disruption or disorganization of the overlying oil droplets.

Specimens taken 1 day after exposure to the laser (Fig. 3) were characterized by marked edema of the nerve fiber layer exterior to the ganglion cell layer and edema of the inner nerve fiber layer. Occasional ganglion cell nuclei were slightly hyperchromatic. The structure of the inner nuclear layer is totally disrupted. The outer limiting membrane disappeared. The nuclei of both the inner and outer nuclear layers were markedly pyknotic. The photoreceptor cytoplasmic elements were absent, leaving an apparent space above the retinal pigment epithelium, which was disorganized and showed elongation of the cell bodies into the overlying space. In some areas the retinal pigment epithelium totally disappeared.

Specimens taken two days after exposure to the laser were similar to those taken one day after exposure, with occasional pyknotic nuclei in ganglion cell layer.

Specimens taken 8 days after exposure to the laser (Figure 4) were characterized by edema and rarefaction of the nerve fiber layers, especially the innermost nerve fiber layer. In some parts of the lesion, edema extended all the way through the inner nuclear layer. In these regions, the nuclei of the inner nuclear layer were slightly hyperchromatic. The outer nuclear layer nuclei were missing in these regions. Macrophages were abundant in the large spaces outside the outer limiting membrane. Integrity of inner and outer segments adjacent to these

FIGURE 2 Section through pigeon retina harvested 1 hour after exposure to an argon laser beam at 7 times the ED_{50} for an ophthalmoscopically visible lesion. Note edema and rarefaction of nerve fiber layers and vacuolation of nuclear layers. (Artifactual wrinkling of the tissue on the slide obscures some features.) Two micron section, original magnification 125×.

spaces appeared to be maintained. There was disruption of the retinal pigment epithelium and the outer limiting membrane was collapsed inwards. There were occasional discrete spaces in the outer nuclear layer at areas away from the area of maximum damage. In areas in which the photoreceptor cells were absent, occasional macrophages were present.

DISCUSSION

Despite the expense and increasing political problems of using mammals, and especially primates, in basic studies of laser-induced damage and its treatment, and despite the ready availability of pigeons, with the well-documented techniques for training and assessing their training, only a very few published studies have used these animals. Marshall *et al.* [5] studied the damage to pigeon retina from moderate continuous light exposure using fluorescent lights. Electron microscopy revealed loss of cone outer segments or vacuolation and glycogen accumulation in the cytoplasmic elements of the photoreceptors. The oil

FIGURE 3 Section through pigeon retina harvested 1 day after exposure to an argon laser beam at 7 times the ED_{50} for an ophthalmoscopically visible lesion. Note edema of nerve fiber layer, disruption of inner nuclear layer, and absence of photoreceptor cytoplasmic elements. Two micron section, original magnification 125×.

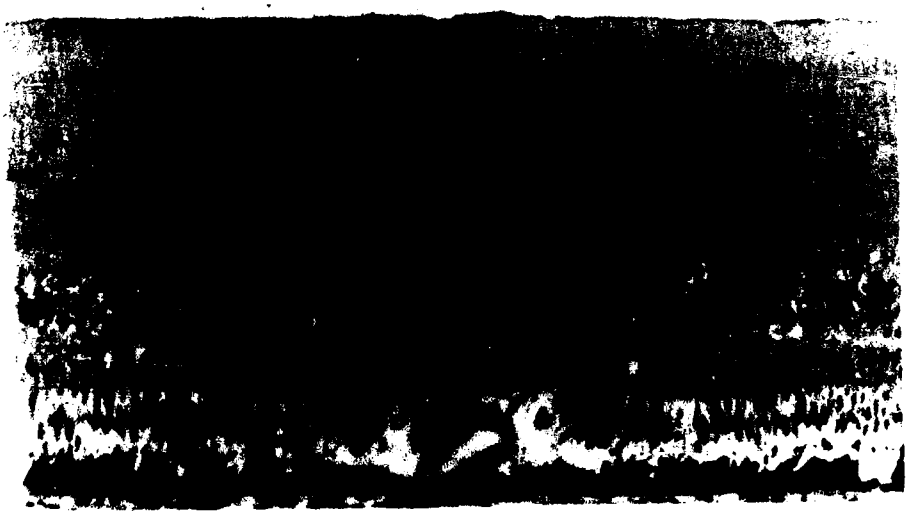

FIGURE 4 Section through pigeon retina harvested 1 day after exposure to an argon laser beam at 7 times the ED_{50} for an ophthalmoscopically visible lesion. Note the edema of the inner nerve fiber layer. This section is from near the edge of the lesion, so the massive damage to the outer retinal layers is not present. However, note the rarefaction of the center part of the nerve fiber layer. Two micron section, original magnification 125×.

droplets of the pigeon retina were apparently not damaged. No damage to cone pedicles or inner portions of the retina was reported. Blough [6] studied visual acuity threshold in the pigeon under a variety of conditions, including in the presence of foveal lesions placed by a ruby laser photocoagulator. Her results confirmed those of several earlier studies in suggesting that the pigeon fovea is relatively unimportant to acuity. No histopathologic evaluation of the foveal lesions was performed.

Conley and Fite [7] studied the effect of laser-induced foveal lesions on optokinetic nystagmus in the pigeon and examined histological sections of regions of the retina in which several closely-spaced lesions had been placed with a ruby laser photocoagulator. The damage in their experimental system was confined to the photoreceptor region and the outer nuclear layer. Energy levels introduced to the eye were about 280 microJoules; the natural history of the lesion was not described.

Spencer [8] studied the sequential clinical and histopathological changes in cynomolgus monkeys produced by lasers operating at 694 nm (a q-switched ruby laser) or at 532 nm (a frequency-doubled neodymium laser) at approximately three times ED50 for an ophthalmoscopically visible lesion. At these levels, the most severely affected parts of the retina were the retinal pigment epithelium and the photoreceptor outer segments. With time, phagocytic cells appeared and began to clear debris from the lesion sites.

The studies presented here demonstrate that the underlying nature of laser-induced retinal lesions is similar anatomically to that of lesions produced in other organisms. Energy levels employed in the present studies did not produce such dramatic lesions as seen in previous studies by Marshall and Mellerio [9–12] and various other investigators, but the similarity is nevertheless appreciable. The oil droplets of the pigeon eye did not serve as an "energy sink" in these experiments. The damage was neither localized nor maximum in the region of the oil droplets. Of course, in those areas in which the photoreceptor cells were absent, the oil droplets had also disappeared. The damage to the eye was apparently most severe in the region between the retinal pigment epithelium and the outer limiting membrane, as seen in studies of cynomolgus monkeys (8). The center of the acute lesion showed greatest damage in and adjacent to the melanin layer, implying that the energy was absorbed by the melanin; evidence of energy absorption by retinal blood or vasculature was not seen. As the lesion matured over several days, the internal layers of the retina were increasingly affected, reflected by increasing edema and changes of nuclear layers. The only sign of any type of recovery during the 8-day period studied was the appearance of macrophages in the damaged areas, first seen in the 8-day sections.

The present study was performed to provide baseline data so that pigeons may be more readily used in the future. The areas and extent of damage caused by the laser devices employed here appear to be similar in nature to those encountered in earlier studies which employed other species, including rabbits and primates. The results obtained here imply that the pigeon can be a useful experimental subject and that, within the limits of our experimental design, the differences in structure of the pigeon retina do not prevent anatomic data obtained in that species from contributing to the understanding of basic mechanisms of laser-induced retinal damage in man.

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Defense. Research was conducted in compliance with the Animal Welfare Act, and other federal statutes and regulations relating to animals and experiments involving animals and adheres to principles stated in the *Guide for the care and use of laboratory animals*, NIH publication 86–23, 1985 edition.

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